THE VIABILITY OF HETEROZYGOTES FOR LETHALS[1]

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IN a preliminary report STERN and NOVITSKI (1948) presented data on the viability of individuals of *Drosophila melanogaster* heterozygous for recessive sex-linked lethals. At that time 33 different lethals had been tested. Since then the data have been enlarged and now embrace a total of 77 different X-chromosomal lethals.

THE LETHALS

All lethals arose in males from the Canton-S stock, a strain of flies which had been made isogenic, by the use of marker and crossover suppressor chromosomes, by C. B. BRIDGES, and had gone through renewed crosses leading to isogeneity in 1939. While no obvious heterogeneity has been encountered in the stock, apart from a very low number of visible mutations and the lethal mutations some of which form the material for the viability tests, it is realized that heterogeneity must have been present in the stock during the course of the experiments. As will be described below the lethals were kept in stocks derived from crosses of the Canton-S males to " Muller-5 " females. These females were not isogenic. All tests were carried out so that heterogeneity in the genetic background was randomized between lethal-carriers and lethal-free controls.

Of the 77 lethals tested for heterozygous viability effects 37 were " spontaneous " from untreated sperm which had been stored in the spermathecae of inseminated females and had served as controls for sperm subjected to ionizing radiations. The other 40 lethals came from sperm which had been irradiated during storage in spermathecae (CASPARI and STERN 1948; UPHOFF and STERN 1949). The total dosage of irradiation administered continuously either over 24 hours or 21 days in the form of gamma rays from a radium needle, amounted to approximately 52.5 r. (The statement in STERN and NOVITSKI (1948) that some of the lethals came from X-ray experiments was in error.) In the experiments of CASPARI and UPHOFF quoted above, the percentage of " control lethals " averaged approximately three-quarters the percentage of lethals obtained in the irradiated series. Therefore, among the " experimental

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lethals" approximately three-quarters must have had in reality the same "spontaneous" origin as the "control lethals." The latter have been designated as *lc,* those from irradiated sperm as *lr.* A specific number accompanying these symbols serves to identify each lethal.

The lethals studied had been defined as lethals by their original discoverers on the basis of the finding that no adult males containing the mutated chromosome had hatched in the first test generation where they had an opportunity of appearing (for a specific exception see CASPARI and STERN (1948) page 79). In the present experiment, as in the earlier ones, occasionally one or a few males with a "lethal" X-chromosome succeeded in developing to the adult stage and appeared as phenotypically normal individuals. As will be explained in the section on "Methods," the finding of normal males among the offspring of F_2 test mothers would result in misclassification of a lethal-carrying mother as being not a carrier. The overall error resulting from such incidences must have been very low in our experiments. During a period of several years of routine transferring and inspecting of the 77 different lethal-carrying stocks, in only seven were "normal" males found and always in a very low frequency. In some of the stocks these "normal" males may have been rare survivors, in others possibly double-crossovers containing the normal allele of the lethal from the "Muller-5 chromosome." There is evidence for occasional survival of "lethal" males since one of the seven lethals referred to permits development through the pupal stage and half-way emergence from the pupa case. There were six additional lethals in which no normal male had been found during transfers of stocks but in which partly emerged males were observed and, in a test culture, an occasional survivor hatched completely. Finally, two more lethals without survivors in the stock and without partly emerged individuals in pupa cases, produced two test cultures with single normal-appearing males, in addition to numerous "Muller-5" males. Altogether 15 out of 77 lethal strains occasionally showed "normal" males, and in seven of these death of lethal males occurred usually in the pupa case. Most of the normal males in the other eight strains may well have been very rare double crossovers.

It may be safely assumed that the great majority of the chromosomes with spontaneous (control) lethals originally carried a lethal change at only a single locus. Similarly, the chromosomes with the lethals which were induced by low dosage irradiation, probably had a change in a single chromosome region only, although some of these may represent changes affecting several adjacent loci. It must be realized, however, that the lethal-carrying chromosomes were kept in stock for considerable periods before being tested for viability. During these times new lethal mutations might have arisen spontaneously; so that lethal-carrying chromosomes may possibly contain more than one lethal.

The frequency of the occurrence of chromosomes with more than one lethal can be estimated. If the stocks had been carried on by means of a single lethal-carrying female per generation, then, assuming a spontaneous mutation rate of sex-linked lethals of .2 percent and an average interval of 50 generations between occurrence of the original lethal event and the viability test, about eight lethal stocks out of 77 would be expected to have accumulated a second lethal. Since the stocks were carried on by means of a rough average of twenty females per generation more than eight lethal stocks had a chance of accumulating a second lethal in some of their X-chromosomes. Random selection of females for continuation of the stock would have subjected this mixture of chromosomes with only the original and with two lethals to drift, and thus may have led to loss of the double lethals in some of the stocks. In others, drift might have led to fixation, while in still others a mixture may have been retained. Altogether, only few stocks would be expected to carry chromosomes with more lethals than the original one.

Linkage tests to localize the lethals in 11 stocks were carried out after completion of the viability tests. As will be described in detail, in ten of these stocks the tests indicate presence of a single lethal. In the eleventh stock the lethal action was associated with an inversion involving a considerable section of the chromosome.

Two of the 77 lethals were found to produce at times a morphological anomaly in adult heterozygous females. These two lethals, *lc 5* and *lr 70,* one of which came from a control and the other from an irradiation experiment, will be treated separately from the main group of 75 lethals.

METHODS

The cultures were raised in incubators kept at 26 ± 2°C. In some tests, however, there were short periods in which the temperature fluctuated beyond these limits. The usual molasses-cornmeal medium enriched with dry brewers' yeast was used for these cultures, and Tegosept-M was used to keep down mold growth. The flies whose viability was tested were females of the constitution " Muller-5 "/*lethal.* " Muller-5 " is an abbreviation for an X-chromosome, synthesized by H. J. MULLER, of the formula sc^{S1} *B In-S* w^a sc^8 (for a detailed description see SPENCER and STERN (1948), page 44). The most important features of the Muller-5 chromosome are its two inversions, one included within the other, which permit very little crossing-over throughout the length of the chromosome, and its markers Bar (*B*) eye-shape and apricot (w^a) eye color which permit phenotypic recognition of the chromosome.

Crosses were made so as to obtain for each lethal, simultaneously, as sibs, Muller-5/*l* and Muller-5/+ flies (the normal chromosome was derived from the same Canton-S stock which had been the source of the lethals). These crosses were as follows (using virgin females unless noted otherwise):

P Muller-5/*l* females (from Muller-5/*l* female × Muller-5 male stock) × + males. Forty or fifty pairs of parent flies were used in each half-pint culture bottle.

F_1 *l*/+ females × Muller-5 males. Forty or fifty pairs of parent flies were used in each half-pint culture bottle. After the parent flies had been in the culture bottle for 4–6 days they were removed. Then a wad of Kleenex or similar paper moistened with a heavy suspension of live yeast in water was partially submerged in the food.

F_2 single females of genotypes Muller-5/*l* or Muller-5/+ × Muller-5 males. These cultures were in one ounce vessels, " creamers."

Depending on the genotype of the F_2 females the F_3 male progeny should consist either of only Muller-5 flies with Bar and apricot eyes (mother, Muller-5/*l*); or of both Muller-5 flies and normal flies with round and red eyes (mother, Muller-5/+).

The two types of F_2 females are phenotypically indistinguishable. Theoretically, they should occur in equal numbers. However, if the heterozygous lethal genotype caused a degree of survival from egg to adult different from that of the homozygous normal, the inequality in numbers of the two types would serve as a measure of the viability effect of the heterozygous lethal. *As an index of viability, the number of cultures from lethal-carrying F_2 females was expressed as a fraction of the number of cultures from lethal-free F_2 females.* This index is equal to one if the viability of the heterozygous lethal is equal to that of the lethal-free females. The index is smaller, or larger than one for lethals with reduced, or increased viability in heterozygotes. Some biological problems regarding the interpretation of viability indexes will be discussed in the section on " The relativity of viability indexes." Statistically, the index as defined above has the disadvantage that chance deviations give an asymmetrical distribution around a true value. Therefore, for various statistical tests to be presented below, a different index has been used, namely the number of cultures from lethal-carrying F_2 females as a fraction of the total number, *i.e.*, of both lethal-carrying and lethal-free F_2 females.

The number of F_2 females tested separately for the determination of the viability indexes varied from 227 to 903 for the initial test of each lethal, with a mean number of 541.8. Sterility reduced the number of successful initial tests to a range from 193 to 874, with a mean of 498.9.

Apart from some of the very earliest experiments care was taken to insure virginity of the F_2 females. Since the brothers of these females included normal males non-virginity could introduce the following source of error: If the female had the constitution Muller-5/*l* and had been fertilized by a normal male, then non-disjunction (of either the primary or secondary type) of her X-chromosome could result in the appearance of normal male offspring and thus would lead to misclassification of the mother as Muller-5/+. No danger of misclassification would arise if the mother had the genotype Muller-5/+. Thus, non-virginity coupled with non-disjunction would have led to an underestimate of the viability of lethal-carrying females.

The initial scoring of the test cultures was usually done between the eleventh and fifteenth day after mating of the parents. If during the inspection one or more red and round eyed males were seen, the F_2 female was classified as Muller-5/+, and the culture discarded. If no red and round eyed male was seen, the culture was reinspected between the fifteenth and twenty-first day after initiation. If again no red and round eyed males were observed although the culture contained a specified minimum number of Muller-5 males, the F_2 female was classified as Muller-5/*l*, and the culture discarded.

An explanation is in order for the term " specified minimum number of Muller-5 males." This number was six in the earlier stages of this investigation but nine later on. This change in specification was due to a change in the number of Muller-5 parental males used per F_2 culture, which first was one but later, for reasons of assuring better fertility, was three. The parent flies were not removed from the cultures before the hatching of the offspring and the large scale inspection of cultures did not make feasible an attempt to classify separately the Muller-5 males into parents and offspring. During the period when a minimum of six Muller-5 males (and no normal males) placed a culture into the lethal-carrying class, these six males may have been either all offspring, or may have consisted of five offspring and one parent. Later, when a minimum of nine Muller-5 males (and no normal males) placed a culture into the lethal-carrying class, these nine males may have been either all offspring or may have consisted of six to eight offspring and three to one parent individuals.

Frequently, no red and round eyed males were found in a culture, but also fewer than the specified minimum number of Muller-5 males were observed. Cultures of this type sometimes were those in which only few offspring had been produced; but more frequently they were cultures in which there were many offspring, but the food had become soft, and in consequence the majority of the flies had died and disintegrated beyond sufficient recognition. Usually no further tests were made with these cultures and they were classified as " doubtful." A considerable number of such cultures, however, was kept for an additional period to permit hatching of more flies including those of a second generation. If normal males appeared, assignment of the culture could be made to the non-lethal-bearing class, while if many Muller-5 males and no normal males developed, assignment to the lethal-bearing class was made. In many of these held-over cultures no normal and not enough Muller-5 males hatched to remove them from the " doubtful " class. In a further attempt to determine the constitution of parent females of doubtful cultures, heterozygous Muller-5 F_3 females were used for new tests. These tests then served to assign the correct constitution, Muller-5/*l* or Muller-5/+, to the original F_2 test female. Some of these tests again were undecisive and could not serve to remove the original test culture from the " doubtful " class. The interpretation of the doubtfuls will be dealt with below.

Finally, some test females produced no offspring at all, due to the early death of themselves or their mates, or to sterility. All these females are listed as " steriles."

ANALYSIS OF DATA

In table 1 the combined results of the 75 initial tests, based on a total of 40,633 females have been summarized. Twenty-two of the lethals were retested one, two, or three times. Since the retests were made with lethals selected for specific properties they have not been included in table 1. A record of all tests is presented in table 2.

Sterile tests

An inspection of tables 1 and 2 will show that the frequency of sterile cultures varied greatly from test to test, and that the average frequency of sterile cultures was higher in tests of lethals from irradiated than from control sperm. These facts are mainly or perhaps exclusively the result of variations in technique. The high frequencies of sterile cultures occurred mostly in the earlier period of the work. From the totals of the earlier initial tests, the percentage of sterile cultures was 14.9. Later, when more parental males per F_2 culture were employed than at the beginning, the average percentage of sterile cultures decreased to 2.4.

The higher frequency of sterile cultures among *lr* lethals than among *lc* lethals is mainly the result of the "accident" that in the earlier period the number of *lr* lethals tested was about double that of the *lc* lethals tested; while

TABLE 1

Combined results of initial tests of 40,633 females, heterozygous for, or free from one or the other of 75 lethals.

No. and source of lethals	Females heterozygous for		Doubtful	Sterile
	Lethal	Non-lethal		
36 from control sperm (*lc*)	9230	9838	184	1013
39 from irradiated sperm (*lr*)	8648	9200	318	2202

in the later period the reverse was true. The mean percentages of sterile cultures in these two periods were 15.7 and 3.5 for *lr* and 13.1 and 1.8 for *lc*.

By their very nature the sterile cultures did not permit assignment of the F_2 females to either the lethal or the normal-containing genotype. As far as the sterility was caused by non-functioning of the males it may be assumed that the group of females remaining without offspring represented a random sample of the population. Some of the sterility encountered must have been due to non-functioning of the females themselves. If these females also were a random sample of lethal-bearing and lethal-free individuals, then the validity of the viability indexes which were calculated without regard to the existence of sterile cultures, is not affected by omission of these cultures. If, however, the sterile cultures contained a disproportionate number of females of one or the other genotype, then the interpretation of the viability indexes would have to be changed. Instead of indicating the relative number of lethal-bearing flies which have reached maturity the indexes would signify the relative number of lethal-bearing flies which have reached maturity and produced offspring.

TABLE 2

Results of viability tests for individual lethals which comprise initial tests, repeat tests, and means. Symbols for females with these X-chromosomes: l = Muller-5/lethal; non-lethal = Muller-5/non-lethal; df = either l or non-lethal and fertile (see text); st = either l or non-lethal and sterile. n = l + df + non-lethal. $\Delta = [(100)(l+df)/n] - (100)(.5n)/n$. $\sigma = 50/\sqrt{n}$. Percent df as calculated for column 4 was based on fertile tests only. The values in column (2) have been adjusted to the nearest first decimal place. Among others, those with an asterisk (*) have been adjusted upward. Values accurate to two decimal places were used in figures 2, 3, and table 5.

Lethal strain	Viability index $\frac{l + df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lc 21	1.312	3.4	351	0.32	6	622
″ ″	1.045	1.3	1850	0.41	39	3650
Mean	1.080	2.5	2201	0.40	45	4272
lc 3	1.258	2.8	320	2.65	25	603
lr 12	1.212	2.3	324	0.34	7	595
″ ″	1.027	0.3	301	0.17	5	596
″ ″	1.141	1.6	314	0.17	10	591
Mean	1.124	2.5*	939	0.22	22	1782
lr 99	1.189	1.8	233	1.36	50	440
″ ″	1.065	0.8	285	1.41	40	568
″ ″	0.947	0.5	192	1.23	76	405
Mean	1.066	1.2	710	1.34	166	1413
lr 98	1.135	1.2	184	4.74	104	380
lr 50	1.119	1.4	311	0	12	589
″ ″	0.890	1.4	282	0	2	599
″ ″	0.866	1.7	272	0	15	586
Mean	0.952	1.0	865	0	29	1774
lr 48	1.109	0.8	136	2.57	213	272
″ ″	0.970	0.3	216	1.97	44	457
Mean	1.019	0.3	352	2.19	257	729
lc 23	1.097	1.1	301	0.52	7	581
″ ″	1.114	1.4	334	1.23	49	649
″ ″	1.120	1.7	463	0.45	19	884
Mean	1.112	2.4	1098	0.71	75	2114
lr 83	1.092	0.9	235	0.66	20	456
lc 10	1.090	1.0	304	0	4	583
″ ″	1.081	0.9	303	0.51	11	589
″ ″	1.143	1.6	312	0	15	585
″ ″	0.921	1.3	474	0.10	13	991
Mean	1.034	0.9	1393	0.15	43	2748

TABLE 2 (*continued*)

Lethal strain	Viability index $\frac{l + df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lr 10	1.085	1.0*	280	2.48	23	565
lc 13	1.077	0.9	301	1.18	23	594
lc 17	1.067	0.8	320	0	10	620
lr 65	1.059	0.7	303	0	12	589
lr 13	1.053	0.5	211	2.10	30	429
lr 44	1.052	0.5	218	0.70	55	431
lc 19	1.052	0.5	193	2.53	53	396
lr 89	1.052	0.4	154	2.83	68	318
lc 66	1.043	0.4	181	2.93	79	376
" "	0.901	1.0*	155	2.33	133	344
Mean	0.973	0.4	336	2.64	212	720
lr 1	1.040	0.5	328	1.80	31	667
lr 80	1.024	0.2	203	3.04	37	427
lc 24	1.021	0.3	286	0.35	18	570
lr 23	1.018	0.2	228	0.22	14	454
lr 97	1.014	0.1	211	0.94	54	427
lr 45	1.007	0.1	130	1.86	217	269
lr 96	1.004	0.0	214	4.67	14	471
lc 27	1.003	0.0	304	0.65	8	615
lc 25	1.000	0	278	1.57	14	574
lr 95	0.995	0.0	209	1.17	57	429
lc 8	0.990	0.1	296	0.17	4	597
lr 3	0.969	0.4	332	1.02	7	689
lr 20	0.964	0.4	177	2.87	81	383
lc 11	0.964	0.4	293	0.17	2	599
lr 6	0.962	0.5*	273	1.23	29	571
lc 86	0.959	0.4	195	4.14	40	435
lr 34	0.955	0.5*	200	2.33	39	430
lr 91	0.955	0.4	162	1.74	132	344
" "	1.035	0.4	233	0.86	20	466
" "	0.993	0.1	142	2.34	180	299
Mean	0.998	0.0	537	1.53	332	1109

TABLE 2 (*continued*)

Lethal strain	Viability index $\frac{1 + df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lr 77	0.953	0.5	214	2.18	19	459
" "	0.702	3.4	155	0.78	97	383
" "	1.140	1.3	196	1.84	111	381
Mean	0.917	1.5	565	1.64	227	1223
lr 53	0.953	0.6	276	1.04	10	578
lr 9	0.949	0.6	260	2.99	29	569
lc 99	0.947	0.6	187	5.94	50	438
lc 98	0.944	0.6	216	0.67	33	451
lc 20	0.944	0.7	301	0.16	6	622
lr 68	0.935	0.7	170	4.63	95	389
" "	1.131	0.8	91	2.23	37	179
" "	0.800	2.2	176	0.99	82	405
Mean	0.908	1.5	437	2.67	214	973
lr 47	0.932	0.8	270	0.35	24	564
lr 2	0.930	0.9	317	0.45	29	664
lc 26	0.929	0.9	297	0.48	6	623
lc 2	0.928	0.8	220	0	10	457
lr 4	0.925	1.0*	303	1.09	46	645
lr 54	0.923	0.7	140	1.33	73	300
lc 9	0.920	1.0	285	0.17	4	597
lc 22	0.925	1.2	413	0.80	29	874
lr 94	0.916	0.9	181	1.29	95	389
" "	0.976	0.2	196	0.99	82	405
" "	0.722	3.3	173	1.41	63	427
Mean	0.861	2.6	550	1.23	240	1221
lc 97	0.911	1.0*	204	0.23	56	430
lc 45	0.911	0.6	90	1.04	34	193
" "	1.033	0.3	179	1.91	124	366
Mean	0.989	0.1	269	1.61	158	559
lc 1	0.910	1.1	283	0	7	594
" "	0.986	0.2	291	0	14	586
Mean	0.947	0.9	574	0	21	1180
lc 6	0.910	0.9	191	0.25	9	403
lr 57	0.908	1.0*	180	1.78	91	393

TABLE 2 (*continued*)

Lethal strain	Viability index $\frac{l + df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lr 73	0.907	1.0	186	4.20	57	429
lc 28	0.898	1.3	287	0.49	16	613
lc 95	0.881	1.4	233	0.60	84	504
" "	1.012	0.1	165	1.48	152	338
Mean	0.931	1.0	398	0.95	236	842
lc 14	0.879	1.6	265	1.70	23	588
lc 57	0.871	1.4	198	0.92	54	434
lc 7	0.865	1.8	273	0.50	4	595
lc 36	0.864	1.8	272	0.34	8	591
lc 12	0.861	1.8	261	0.87	13	575
lr 93	0.854	1.5*	158	1.69	120	356
" "	0.920	0.9	197	2.31	70	432
Mean	0.890	1.6	355	2.03	190	788
lr 81	0.851	1.6	174	0.78	75	385
" "	0.890	1.3	201	3.87	50	465
" "	0.922	0.8	194	1.67	36	419
Mean	0.888	2.1	569	2.21	161	1269
lc 62	0.840	1.9	220	1.01	94	493
" "	0.936	0.7	184	1.28	99	391
Mean	0.881	1.9	404	1.13	193	884
lc 96	0.838	1.8	173	3.19	96	408
lr 92	0.837	1.8	165	6.00	69	417
" "	0.917	0.9	217	0.87	41	462
Mean	0.878	1.9	382	3.30	110	879
lr 78	0.833	1.9	187	1.86	39	429
" "	0.844	1.7	187	1.88	53	426
" "	1.052	0.6	260	0.97	44	517
Mean	0.914	1.7	634	1.53	136	1372
lc 64	0.823	2.0*	179	1.70	74	412
" "	1.031	0.3	197	0.76	73	394
Mean	0.919	1.2	376	1.24	147	806
lc 4	0.778	3.0	259	0	10	592
lr 30	0.602	6.0*	211	0.87	25	575
" "	0.666	4.8	230	0.17	22	578
Mean	0.633	7.6	441	0.52	47	1153

TABLE 2 (*continued*)

Lethal strain	Viability index $\frac{l + df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lc 5	0.665	4.7	211	1.98	45	556
" "	0.613	5.9	224	0.99	92	605
Mean	0.638	7.5	435	1.46	137	1161
lr 70	0.452	8.2	145	0.21	14	469
" "	0.490	8.3	195	0	9	593
Mean	0.473	11.7	340	0.09	23	1062

This interpretation of the viability indexes was found to be the more accurate one for at least two of the lethals, one of which belongs to the lethals with morphological effects in heterozygotes. During the localization tests and viability tests, it was found that *lc 3* and *lc 5* showed higher percentages of sterile females than did the other lethals which were tested at the same time. A direct test for *lc 3* showed that while 1000 Muller-5/*lc 3* females gave 10.7 percent steriles; 1000 Muller-5/+ females gave .6 percent steriles. At the same time other tests were made in order to find out whether the sterility and lethal factors were localized in the same region of the chromosome. For this purpose Muller-5/*lc 3* females with an X-chromosome derived from two types of stocks were used. In one type of stock the left end containing the lethal came from *lc 3,* and the right end from Canton-S. The junction point was between the lethal locus (about 1.6) and *cv.* One thousand Muller-5/*lc 3* females from ten strains with such an X-chromosome gave 4.1 percent steriles; while 1000 Muller-5/+ females from six strains with the complementary chromosome containing no lethal, a left end from Canton-S, and a right end from *lc 3,* gave .9 percent steriles. From these results the lethal and sterility factors for *lc 3* seem to be closely associated if not identical. In the case of *lc 5* the high percentage of steriles found was shown to be mainly due to the death of about 25 percent of the females 0–2 days after emergence. This lethal is described further in the section, " Lethals with morphological effects in heterozygotes."

In addition to this evidence that lethals could increase the percentage of sterile Muller-5/*l* females, it was also shown that the percentage of steriles for some lethals could be as low as the control rate of about .6 percent, for ten lethals gave less than one percent steriles in at least one test. And since the best comparison of percentage steriles in controls and lethals must be made from tests run simultaneously, many other lethals with percentages higher than one percent sterile may not have exceeded their unknown control rate.

Whatever the distribution of the steriles in regard to the two genotypes, the index of lethal-bearing fertile to lethal-free fertile females gives a measure of the reproductive fitness of the lethal heterozygotes (assuming, until further study, that the fecundity of the two genotypes is alike).

Doubtful tests

The " doubtful " tests pose a more serious problem than the sterile tests. As described under " Methods " this group of tests is made up of fertile cultures in which no normal males were found but in which the number of Muller-5 males was very small. Such a small yield of males leaves considerable uncertainty as to whether the absence of normal males is due to the lethal-carrying nature of the non-Muller-5 chromosome in the parent female, or whether the female was Muller-5/+ and, by chance, produced only Muller-5 sons. Arbitrarily, cultures with less than six (or, less than nine) Muller-5 males and no normal males were defined as doubtful. This definition introduces an error and opens a question. The error consists in the fact that there is still an appreciable chance for Muller-5/+ females to produce six or more Muller-5 and no normal sons and thus be misclassified as lethal-bearing. If the viability of a Muller-5 male zygote were equal to that of a normal male zygote the chance of a Muller-5/+ female producing six Muller-5 males and no normal males would be $(1/2)^6$ or about 1.6 percent. In reality, counts based on 3655 males from Muller-5/+ females show that under " normal " culture conditions—*not* necessarily typical of doubtful cultures—the viability of Muller-5 males was only 87 percent of that of normals so that the chance of misclassification in the case of six Muller-5 males is reduced to $(.87/1.87)^6$ or about 1.0 percent. With more than six Muller-5 males, and no normal males, the danger of misclassification of Muller-5/+ females becomes exponentially smaller, being $(.87/1.87)^n$ where n = number of Muller-5 males. Since only a small fraction of any test series—estimated as never more than 1.5 percent and usually very much less—fell into the class of six or more, or respectively nine or more Muller-5 males, and no normal males, the error of misclassification of normal-carrying females is negligibly small.

The question which is opened by the definition of cultures with less than six, or less than nine, Muller-5 and no normal males as " doubtful " is the following: What fraction of the doubtful tests should be assigned to the Muller-5/*l* and what fraction to the Muller-5/+ class? This question may be approached from two different angles, the theoretical and the empirical. Theoretically, it can be calculated how many cultures with 0, 1, 2, 3, 4, and 5, etc., Muller-5 males and no normal males would belong to one or the other of the two classes. Obviously, the chance of a culture with no normal males belonging to the Muller-5/*l* and not to the Muller-5/+ type would increase with increasing number of Muller-5 males present. Therefore, the relative numbers of cultures with from 0 to 8 Muller-5 males would be a determining factor in calculating the over-all partition of the doubtful tests into the two categories. More specifically, however, the calculation would not only depend on the number of Muller-5 males present in a culture, but also on other factors. The partition of the doubtful culture group would depend on such factors as (1) the " true " ratio of lethal and non-lethal-bearing cultures in each lethal test, (2) the proportion of doubtful creamers which was due to low egg laying activity of the F_2 female, (3) the proportion of doubtful creamers which was due to the death and disintegration of most of a large number of adults because of unfavorable conditions before the creamer was to be checked, (4) possible selective factors in adult death, (5) possible differential effect of the presence or absence of the lethal F_3 genotypes on the conditions which give doubtful creamers. Since little is known about these various factors, tests were made in order to determine directly how many cultures classified as doubtful in the main test belonged to each of the two genotypic classes. Five such tests are available (table 3). Test group 1 consisted of 620 doubtful cultures which were simply kept for an additional number of days so as to permit hatching of further flies usually including F_4 individuals. In 220 cultures so few flies were observed that these tests remained in the doubtful class. In the other 400 cultures sufficient flies appeared to assign 365 cultures to the lethal-carrying and 35 to the non-lethal genotypes.

TABLE 3

Results of progeny tests of doubtful cultures.

Test group	Total retests	Original F_2 female		Remained doubtful	%	%
		Lethal-bearing	Non-lethal-bearing		$\frac{l}{l + \text{non-lethal}}$	$\frac{l + df}{\text{total}}$
		l	non-lethal	df		
1	620	365	35	220	91.3	94.4
2	202	193	3	6	98.5	98.5
3	116	109	4	3	96.5	96.6
4	67	61	6	0	91.0	91.0
5	201	191	9	1	95.5	95.5
Totals and means	1206	919	57	230	94.2	95.3

In test groups 2 to 5, heterozygous Muller-5 F_2, F_3, F_4, or F_n females from doubtful cultures were mated to Muller-5 males in order to test for production of normal males. Corresponding to the genotype of the non-Muller-5 F_2 females the non-Muller-5 females in any one of the doubtful cultures all had to be either Muller-5/*l* or Muller-5/+, and the absence or presence of normal sons in the new cultures could thus serve for final identification of the until then doubtful tests. As seen from table 3, some of the progeny tests yielded again so few Muller-5, and no normal males that the cultures still remained doubtful. The great majority of the tests were successful. Excluding those cultures which had remained doubtful, from 91.0 to 98.5 percent were assignable to the lethal-bearing genotype, with an average of 94.2 percent. If one assumes that the cultures which had remained doubtful were all lethal-bearing then the total percentages of the lethal-bearing genotypes varied equally between 91.0 and 98.5. It is probably true, that 91.0, the lowest percent, is an underestimate and 98.5, the highest percent, an overestimate of the true frequency of lethal-bearing cultures among the initially doubtful cultures of which the great majority were never tested further. It is obviously an even greater overestimate to assume that 100 percent of the initially doubtful cultures are lethal-bearing.

In the following sections use will be made of the two extreme estimates, 91 and 100 percent. These two estimates will be employed in connection with different analyses and in each case that estimate will be chosen which leads to the more conservative interpretation of the relevant data.

Tests with less than one percent doubtful cultures

Among the 75 initial test groups of the lethals there were 35 in which the fraction of doubtful cultures amounted to less than one percent of each test group. The mean percentage for 24 control lethals, *lc*, was .36; for 11 irradiated lethals, *lr*, was .45; and for the total was .39. This set of 35 test groups might be regarded as particularly reliable. Assuming that all (100 percent) doubtful cultures were lethal-bearing, the viability indexes (lethal + doubtful/non-lethal) of the 35 lethals range from 1.312 to .602. The mean viability index calculated from the sum of all lethal-bearing (plus doubtful) and all lethal-free cultures is .9579 (table 4, A). Based on 19,471 cases this mean index represents a highly significant decrease from the viability of the non-lethal-bearing females.

TABLE 4

Mean viability indexes based on the sums of: A. 35 initial tests with less than one percent doubtful cultures; B. 40 initial tests with more than one percent doubtful cultures; C. all 75 initial tests. P, based on a chi-square test, signifies the probability that the deviation of the index from one is a chance deviation. 100 percent of doubtfuls assumed to be lethal-carrying.

Test group	Type of lethal	Viability index $\frac{\text{lethal + doubtful}}{\text{non-lethal}}$	n	P
A	24 strains of lc	.9509	13,742	.003
	11 strains of lr	.9748	5,729	.35
	Total	.9579	19,471	.003
B	12 strains of lc	.9721	5,510	.29
	28 strains of lr	.9744	12,437	.16
	Total	.9737	17,947	.08
C	36 strains of lc	.9569	19,252	.002
	39 strains of lr	.9746	18,166	.09
	Total	.9654	37,418	< .001

Homogeneity tests for sums of tests A versus tests B: lc, P = .49; lr, P = .99; totals, P = .44. Homogeneity tests for sums of lc versus lr: A, P = .44; B, P = .92; C, P = .39.

A separation of the 35 lethals into those derived from control males and those from irradiated males yields .9509 mean viability for the sums of tests of the 24 control lethals, and .9748 percent for those of the 11 irradiated lethals. The deviation of the first value from one is highly significant, but that of the second value is not. There is, however, no significant difference between the mean values for the two subgroups ($P = .44$).

It may be concluded that the presence of a lethal, in heterozygous condition, reduces the viability of a female by several percent. This conclusion is based on using tests with a minimum number of doubtfuls and assuming that all doubtfuls were lethal-carriers.

The viability indexes as given in table 4 are calculated on the assumption that the lethals are a homogeneous group with equal effect on viability of heterozygotes. This assumption permitted use of the simple statistical tests which showed the significance of a decrease in viability for heterozygotes. It will be shown below that the lethals are not a homogeneous group in respect to viability, thus confirming the finding of a heterozygous effect. The mean decrease of viability of the different lethals will be shown to be similar to that represented by the indexes of table 4.

Complete tests

In table 4, C, a summary is given of all 75 initial tests regardless of the frequency of doubtful cultures. These 75 tests include the 35 tests treated in the preceding section. The mean percentages of doubtful cultures were as follows: 36 *lc*, .96 percent; 39 *lr*, 1.75 percent; total, 1.34 percent. Assuming again that all doubtful cultures are lethal-bearers, it is seen in table 4, C, that the mean viability indexes for the total and the *lc* and *lr* groups are very similar to those for the partial data presented in table 4, A. The decreases in viability from one for the total and the 36 *lc* tests are again highly significant, that for the 39 *lr* tests only at the nine percent level. There is no significant difference between the means of the *lc* and *lr* groups ($P = .39$).

The similarity of the mean indexes for the total of 75 lethals and those for the 35 selected lethals is, of course, partly due to the fact that the latter are included in the data for the whole set of lethals. However, the mean indexes for the 35 lethals with less than one percent doubtful tests are also very similar to those of the 40 lethals with more than one percent doubtful tests (table 4, B). Since these mean indexes are so similar to each other it is concluded that the assignment of all doubtful individuals to the lethal-carrier class gives a close approximation to reality. Therefore further analysis will be based on all 75 lethals.

The viability index for the total, .9654, signifies an average decrease of viability for a heterozygous lethal-carrier of approximately 3.5 percent.

The distribution of viability indexes

The decrease of viability of lethal-carriers is not caused by one or a few specific lethals with strong effects in heterozygotes, but is a characteristic of the group of lethals as a whole. This will be demonstrated by a consideration of the distribution of the 75 viability indexes from initial tests.

Figure 1 gives the distribution in terms of the indexes themselves. They vary from .602 to 1.312. If the presence of a lethal in heterozygous constitution would not affect the viability of the carriers there should be an equal number of observed indexes which are larger and smaller than the standard value of one. If only one or a few lethals were responsible for the mean decrease of viability the expected equality of indexes above and below one should not be greatly disturbed except for a surplus of one or a few extreme low deviants. However, inspection of figure 1 shows a general shift of the population of lethal indexes toward less than one with a peak in the .925–.974 class.

Both control and irradiated lethals share in this shift and show very similar distributions; although the indexes for the irradiated lethals show a slight shift to higher values than those for the control lethals. A statistical test for determination of the significance of this apparent difference was made by com-

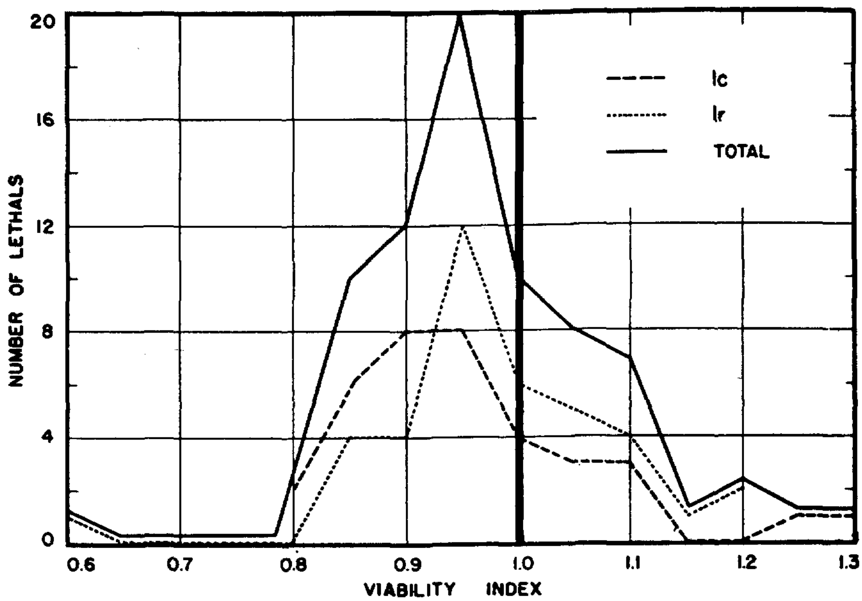


FIGURE 1.—Distribution of viability indexes from 75 initial tests. 100 percent of doubtfuls assumed to be lethal-carrying.

bining the indexes for the *lc* and *lr* lethals each in four classes (1) those below .875, (2) .875 through .974, (3) .975 through 1.074, and (4) 1.075 and above. The chi-square for homogeneity is 1.79 which with three degrees of freedom corresponds to a P value of about .6. Thus, the two distributions do not seem to differ significantly from each other, and the *lc* and *lr* lethals will not be treated separately henceforth. The shift of the distribution of the 75 lethals toward a mean of less than one is statistically significant. This may be shown in various ways.

A simple test consists in comparing the observed number of lethal indexes above and below one with the expected number if one were the mean of the indexes. Obviously, chance expectation would assign equal numbers of lethals to the two classes, namely 37.5. Actually 47.5 lethals gave indexes below and

27.5 above one. Chi-square is 5.33, corresponding to P = .02, strongly indicating significance of the uneven distribution around one.

More detailed tests were based on the distribution of the lethals in terms of standard deviations from an expectation of one (table 5). The data show again a striking shift of the distribution of indexes toward values of less than one. Chi-square tests were performed with several sets of grouped data. In one of these, ten classes were distinguished with intervals of .5 σ except for bracketing together all classes with deviations of $+2\sigma$ and greater and -2σ and greater: $\chi^2 = 25.05$, d.f. = 9, P = .005. Another test was based on five classes, comprising deviations of -1.5σ and greater, -1.49σ through $-.5\sigma$, $-.49\sigma$ through $+.49\sigma$, $.5\sigma$ through 1.49σ, and 1.5σ and greater: $\chi^2 = 17.57$, d.f. = 4, P = .003. In the two preceding tests the smallest expected classes were 3.31 and 5.02, respectively. In order to avoid an overestimation of significance, a third test was made in which all classes with deviations of + 1.0 and greater, and − 1.0 and greater were bracketed together, thus making all class frequencies greater than 11. This grouping still yielded: $\chi^2 = 14.28$, d.f. = 5, P = about .015. Thus, the tests leave no doubt that the reduced viability of lethal-carriers is an attribute of the group of lethals as a whole and not of a few exceptional ones, particularly since the two lowest indexes deviating respectively 3.04 σ and 5.96 σ from expectation had not been given their due weight but been merged with less extreme indexes in each one of the different tests.

TABLE 5

Distribution of viability indexes from 75 initial tests in terms of standard deviations from an expected value of one. 100 percent of doubtfuls are assumed to be lethal-carrying. n = lethal + doubtful + non-lethal; $\sigma = 50/\sqrt{n}$; $\Delta = [(100)(\text{lethal} + \text{doubtful})/n] - (100)(.5n)/n$. The expected numbers of lethals are based on the normal frequency distribution.

$\frac{\Delta}{\sigma}$	0.00–0.49	0.50–0.99	1.00–1.49	1.50–1.99	2.00–2.49	2.50–2.99	3.00–3.49	3.50–3.99	4.00–
Observed positive	9.5	10	4	1	1	1	1	0	0
Observed negative	9.5	18	8	10	0	0	1	0	1
Expected	14.36	11.23	6.89	3.31	1.24	0.37	0.08	0.02	0

On the other hand the tests do not exclude the possibility that not all lethals decrease the viability of carriers. Actually with respect to their viability indexes the 75 lethals do not behave as a homogeneous group. A homogeneity test, with 74 degrees of freedom, yields a chi-square value of 130.31 which corresponds to a P value of less than .001.

In the preceding sections mean viability indexes were calculated from the sums of all lethal and non-lethal-carrying females of the different lethals, but in view of the heterogeneity of the different lethals in their viability effects the best estimates for the effect on viability would be provided by the means of the indexes. Calculating the means of the indexes " lethal-carriers/total " and expressing the results in terms of "lethal-carriers/lethal-free" these indexes were .9526 for 36 *lc*'s, .9750 for 39 *lr*'s and for the total of 75 lethals, .9642.

These indexes are based on the limiting assumption of 100 percent doubtful cultures being lethal-carrying. Using the most likely assumption of 94 percent doubtful cultures being lethal-carrying (table 3) the average index for all 75 lethals becomes .9607. It signifies an average decrease of viability by 3.9 percent per lethal.

Viability indexes over one

Among the 75 viability indexes obtained in the initial tests there were 27.5 which were higher than one. These high values could be caused by any one

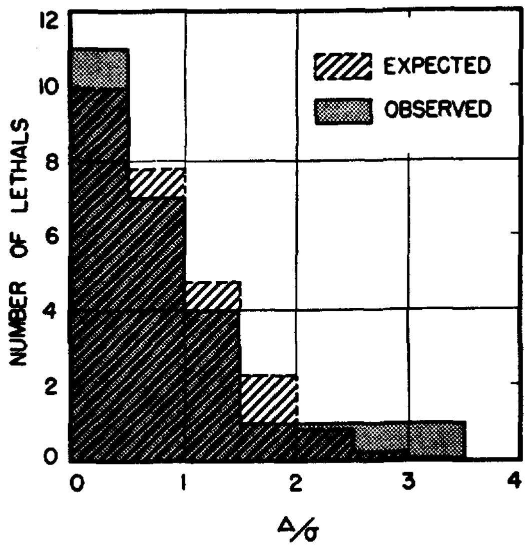


FIGURE 2.—Distribution of (lethal + doubtful)/n indexes from 26 initial tests with (lethal + doubtful)/non-lethal indexes greater than one. Distribution in terms of standard deviations from an expected value of one. 91 percent of doubtfuls are assumed to be lethal-carrying. $n = \text{lethal} + \text{doubtful} + \text{non-lethal}$; $\sigma = 50/\sqrt{n}$; $\Delta = [(100)\ (\text{lethal} + \text{doubtful})/n] - (100)\ (.5n)/n$. The expected numbers of lethals for each class are based on the normal frequency distribution.

of the following phenomena: (1) chance deviations from true values lower than one, (2) chance deviations from true values of one, (3) occurrence of true values higher than one.

It can be expected *a priori* that some of the higher-than-one values are due to phenomenon (1) but the question arises whether this phenomenon can account for all these values. In order to be on the safe side the indexes over one were recalculated using the assumption that only 91 percent of the doubtful tests actually belonged to the lethal classes. This recalculation reduces the indexes slightly and removes 1.5 out of the 27.5 indexes from the group over one, leaving 26 such values. The distribution of these values in terms of their standard deviations from one is given in figure 2 together with a distribution based on an expected value of one. Inspection of the figure shows a general shift of the observed distribution, as compared to the expected, toward the left, that is toward a mean of less than one, except for the three indexes which in terms of the standard deviations deviate the most from one. Their observed frequencies, one for each, are higher than the expected frequencies .86, .25, and .06, respectively.

The difference between the three extreme viability indexes and an index of one are, respectively, 3.35, 2.69, and 2.32 times their standard errors. The probability of obtaining, in a sample of 26 indexes three or more which deviate as much or more than 2.32 σ is .015. It must be stated that this determination of probability is biased in two opposite ways. On the one side, by making, *a posteriori,* 2.32 σ the limiting deviation, P tends to be too small; on the other side, by not giving full weight to the two deviations 2.69 σ and 3.35 σ, P tends to be too large. It is not clear how far these two opposing tendencies balance each other, but it seems probable that the 26 indexes over one include indexes whose true value is greater than one. Further evidence for this contention will be presented in the following sections.

The distribution of the 26 indexes is interpreted to mean (a) that the shift toward less than the expected values signifies the presence of indexes with true values lower than one; (b) that the observation of extreme, high values in greater numbers than expected signifies the occurrence of true values higher than one. These two interpretations assume the existence of phenomena (1) and (3) listed in the first paragraph of this section. Since the indexes of the 75 lethals are distributed over a wide range there is no reason to exclude the additional existence of phenomenon (2), namely of indexes with a true value of one. In other words the class of 26 values over one is regarded as a composite of true values below, at, and above one. The proportions of these three groups are unknown, particularly of the most interesting one, that with better than normal viability.

Retests

General. The viability indexes of 22 of the 75 different lethals were determined in more than one test. As seen in table 2, single retests were carried out for 11, two retests each for ten others, and three retests for the remaining lethal.

The lethals chosen for retests cover the whole range of viability indexes (table 2). However, they do not form a random sample since a deliberate selection was made giving some preference to lethals with extremely low and high indexes. Homogeneity tests (table 6), for each group, of initial tests and

TABLE 6

Probabilities that initial and repeat viability tests for individual lethals were homogeneous. There were two to four tests for each of 22 lethals.

P	> .5	.49–.10	.09–.05	.049–.010	< .01
No. of lethals	5	13	2	0	2

single or multiple retest, show that 20 of the 22 lethals gave results compatible with the assumption that the variations of viability indexes within each group were due only to chance ($P \geqq .05$). Two lethals gave homogeneity probabilities of less than one percent: *lc 21*, P = .0096; *lr 77*, P = .006. The causes for these heterogeneities are unknown. They may involve the presence of a second lethal in some of the tested chromosomes (see page 438). In the case of *lc 21* they may be related to the peculiar emergence pattern of the heterozygous lethal females (see page 441). In addition to the 22 morphologically recessive lethals, retests of the two lethals with dominant morphological effects, which will be discussed below, both gave homogeneity probabilities of > .5.

On the whole, the homogeneity of retests of 20 out of 22 lethals shows the reproducibility of the results. These facts deserve special mention since many retests were carried out by different ones of the authors than those who made the initial or a preceding retest. For six of the 11 lethals with two tests each, the second test was carried out by different workers than those who did the first; for four of the ten lethals with three tests each, different workers were in charge of each of the three tests and for four of the ten lethals with three tests each, one of the tests was carried out by a different worker than the one responsible for the other two. The remaining eight retest groups had no alternation of workers. It may be added that often a period of many months or even several years separated a retest from an earlier determination.

Retests of lethals with viability indexes below one. Among the 22 retested morphologically recessive lethals, 14 had decreased viability for both the initial test and the mean of all tests. The result of homogeneity tests within each of 13 out of 14 groups of retests shows the reality of the deleterious effect of these lethals in heterozygotes.

Retests of lethals with viability indexes over one. Eight of the 22 retested morphologically recessive lethals initially had increased viability. When the average indexes were calculated for each of the eight groups of repeated tests, regressions became apparent which removed two lethals from those with an index over one. Five of the six remaining lethals, *lr 48, lc 10, lr 99, lc 23,* and *lr 12* had average indexes of 1.011, 1.034, 1.061, 1.109, and 1.123 respectively (91 percent doubtfuls = lethals). The differences between these values and one are respectively .15, .86, 1.11, 2.38, and 2.45 times the standard error calculated for an expectation of one. Clearly the estimates for the first three of the five lethals do not differ significantly from one. The last two (*lc 23* and *lr 12*) are greater than one at the .0174 and .0142 levels of significance, respectively.

The mean viability index for the sixth lethal, *lc 21*, was 1.078 (91 percent doubtfuls = lethals) which is 2.46 times the standard error (P = .007). As stated earlier, the initial test and the retest of *lc 21* differed significantly from each other. It seems safer, therefore, not to make use of the initial test of 622 females whose homogeneity had not been studied, but to use the second test only. It comprised 3650 females which consisted of 12 groups derived from 12 single lethal-carrying females, which came themselves from a single mother. The results of the 12 sub-tests were highly compatible with each other (χ^2 for homogeneity = 7.64; d.f. = 11; P = .74). The index for the second test was 1.043, which is 1.28 times the standard error (P = .10).

The values of significance for *lc 23* and *lr 12* cannot be taken as proof of the existence of viability indexes over one. They are the extreme deviants in a population of 24 indexes over one which are composed of the 18 indexes from initial tests which were not repeated and the six average indexes greater than one in repeated tests. The deviations of the 24 indexes from one, in terms of their standard deviations are distributed as shown in figure 3. This distribution differs from the one shown earlier for the 26 initial indexes over one in that it accentuates the deviations of the observed distribution from that ex-

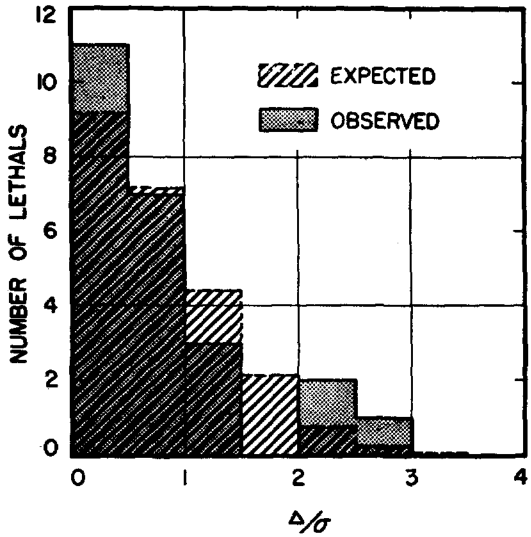


FIGURE 3.—Distribution of (lethal + doubtful)/n viability indexes from 24 complete tests with (lethal + doubtful)/non-lethal indexes greater than one. Distribution in terms of standard deviations from an expected value of one. 91 percent of doubtfuls are assumed to be lethal-carrying. n = lethal + doubtful + non-lethal; $\sigma = 50/\sqrt{n}$; $\Delta = [(100)$ (lethal + doubtful)/n] − (100) (.5n)/n. The expected numbers of lethals for each class are based on the normal frequency distribution.

pected: the shift to the left, of the bulk of the observed indexes, has increased slightly while the group of three indexes deviating from one by more than 2σ has remained. The changes resulted in a discontinuity so that the class deviating from one by 1.5–1.99 σ is not represented at all. This accentuation of heterogeneity, after the retests, in the group of indexes over one is to be expected if this group consists of true indexes (a) below or (b) equal to one on the one side, and (c) above one on the other. Retests within sub-groups (a) and (b) would shift the average indexes to the left, including removal of some of them from the group over one, while retests within sub-group (c) should retain their status as deviating more than by chance from one.

The deviations of the three extreme indexes *lc 23, lr 12,* and *lc 3,* in terms of σ, are 2.38, 2.45, and 2.69. The probabilities of deviating as much or more from one are .0174, .0142, and .0072, respectively, among indexes greater than one. Testing for the probability of obtaining, among 24 indexes over one, three or more which deviate by as much as or more than an amount corresponding to a probability of .02, one obtains a P value of .012. In this determination of P the *a posteriori* limit of .02 has been set considerably above the least empirical deviate chosen with its probability of .0174, and no sufficient weight is given to the greater deviates. It is, therefore, believed that the treatment establishes the existence of viability indexes over one.

The data are weighted against finding true indexes over one. The retests of lethals with initial indexes over one included only one lethal with an initial deviation of less than .67 σ as against seven lethals with a deviation of more than .67 σ. Had more of the slightly deviating lethals been retested the number of final indexes over one would probably have decreased to considerably less than 24 without affecting essentially the frequency of extreme indexes. In addition all indexes over one are based on the underestimate of only 91 percent of doubtful tests belonging to the lethal class. A higher estimate would have raised the indexes.

Before discussing the implications of the establishment of increased viability indexes for lethal-heterozygotes, a possible operational, as opposed to an intrinsic, cause for their occurrence will be dealt with.

The problem of two linked lethals

The rather long period of time over which the lethal-carrying stocks were kept, before and during the viability tests, provided an opportunity for new lethals to arise spontaneously in the lethal-bearing X-chromosomes. The consequences of the presence of more than one lethal in an X-chromosome on the viability indexes require evaluation. The discussion will be restricted to consideration of two lethals in a chromosome.

Assume the presence of two lethals, l^1 and l^2, in one chromosome and the presence of the normal alleles in the other X-chromosome of an F_1 female from the cross Muller-5/l females times normal males. Let this $l^1 l^2/++$ female be mated to a Muller-5 male. From this mating the following types of female offspring will be obtained:

(a)	non-crossovers	$l^1 l^2$/Muller-5, ++/Muller-5
(b)	crossovers	$l^1 +$/Muller-5, $+ l^2$/Muller-5

Let p be the recombination value for l^1 and l^2, v the mean of the true viability indexes for each lethal when present separately, and cv the heterozygote viability of the two lethals if present together. Then the frequencies of the two non-crossover types will be $cv(1-p)/2$ and $(1-p)/2$ respectively, and the frequency of the sum of the crossover types vp. Since the viability tests as carried out in the present investigation can distinguish between two classes only, namely, (1) the three genotypes with one or two lethals, and (2) the lethal-free genotype, the following "apparent" viability index, v′, is calculated from the tests:

$$v' = \frac{cv(1-p) + 2vp}{1-p} = v\left(c + \frac{2p}{1-p}\right) \quad (1)$$

Thus, the apparent viability v' is a function of three parameters, namely, (1) the mean true viability of the two lethals, v, (2) the coefficient, c, which designates the relative viability of the double heterozygote, and (3) p, the recombination value of the two lethals. Perhaps the most interesting fact which the formula expresses is that the apparent viability v' is larger, by the addition of $2vp/(1-p)$, than the true viability cv of the double heterozygote. Since $2p/(1-p)$ increases with increasing values of p, the value of the apparent viability may rise over one independently of the true value.

These relations may be explored further by finding the "critical" recombination value p' at which the apparent viability v' becomes one. It follows from formula (1) that for any value of $p < p'$, v' remains below one, but for any value of $p > p'$, v' rises above one. Substituting $v' = 1$ in (1):

$$p' = \frac{1 - cv}{1 - cv + 2v} \quad (2)$$

This relation is plotted, in figure 4, for a family of curves selected for various values of the true viability v. To illustrate, the line for $v = .961$ shows that at $c = 1.04$ two lethals must be absolutely linked ($p' = 0$) in order to give $v' = 1$, while at $c = 0$ (= lethality of the double heterozygote), p' is about 34 percent. For $c = .9$, p' becomes 6.23 percent and for $c = .5$, p' is 21.04 percent. Or, to choose a much lower true viability $v = .5$, it is seen that for $c = 1$, p' is 33 percent; for $c = .5$, p' is 42.9 percent; and for $c = 0$, p' is 50 percent.

The bearing of these deductions on the results of the viability tests reported in this paper is manifold and depends on the empirical facts regarding v and c. It is reasonable to assume that the majority of the lethal stocks studied contained only a single lethal, and that the true heterozygote viability of most lethals taken singly lies between 1 and .7 with an average of .961 as determined from the tests. It may be assumed further that interaction effects between two lethals in heterozygous state are relatively small and, if present, usually toward lowered viability of the double heterozygote. This places c at values of the order of the true viability of that lethal of a pair which has the lower viability of the two. If c is taken as .7 it appears that for the range of v from 1 to .7 an apparent viability over 1 will be found whenever the lethals are located far enough away from each other to give recombination percentages over 13 to over 26.7.

Having dealt with the critical apparent viability $v' = 1$, we may now consider an extreme case of low apparent viability, $v' = .6$. For this value equation (1) yields:

$$p'' = \frac{.6 - cv}{.6 - cv + 2v} \quad (3)$$

For $p > p''$, v' would be larger than .6. Choosing a value of $v = .9$ it can be seen that p'' approaches 0 when $c = .66$ and rises to 7.7 percent when $c = .5$. Choosing $v = .7$, p'' approaches 0 when $c = .85$ and rises to 15.2 percent when $c = .5$.

The various relations outlined in this theoretical treatment suggest the need for localization data on the lethals. Theoretically any one of the lethal stocks

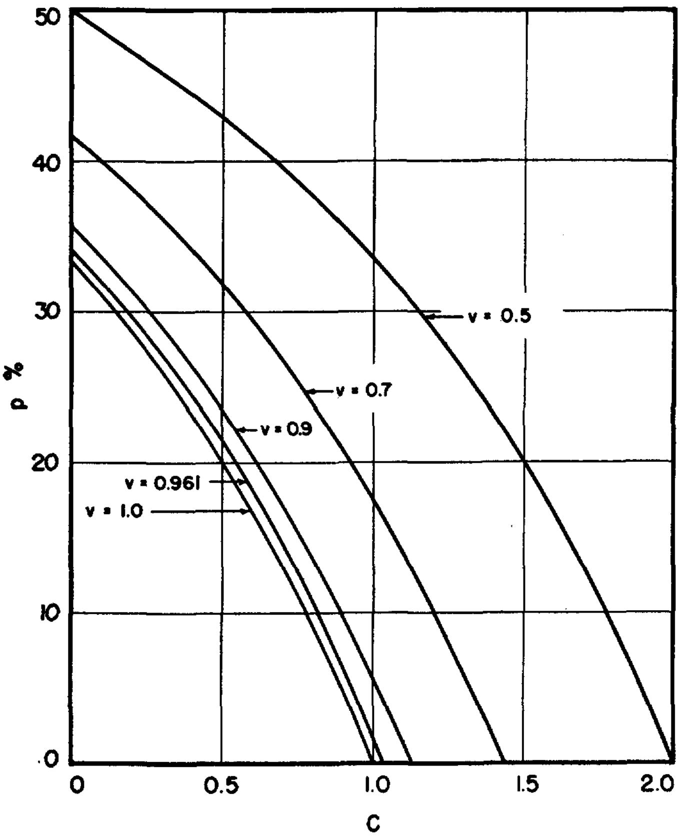


FIGURE 4.—Relation between the apparent viability index, v', and the recombination frequency, p, for two linked lethals with five different values of v, the mean of the true viability indexes for each lethal when present separately: $c =$ coefficient of joint heterozygote viability of two lethals. $cv =$ true viability index for lethals when present together. For given values of v and c the associated value of p represents that recombination frequency at which $v' = 1$. For larger p, $v' > 1$; for smaller p, $v' < 1$.

might contain two lethals in its tested X-chromosome and *a priori* such double lethals might be expected to be distributed at random over the 75 stocks. It has been shown, however, that such a random distribution might result in a nonrandom distribution of the observed viability indexes: On the one hand, low coefficients c for double heterozygotes may yield low apparent viability indexes if the two lethals are located close to one another; and on the other hand, high apparent viability indexes over one may result if the two lethals are located far away from one another. This latter rather unsuspected deduction is of particular importance since it has a bearing on the interpretation of the observed viability indexes over one.

Localization tests

In view of the theoretical considerations regarding the problem of linked lethals 11 lethal chromosomes were selected for localization experiments: namely, (a) five with high viability indexes, (b) three with low viability indexes, and (c) three with intermediate viability indexes. In a first set of tests all lethals were localized with respect to the four genes y (0.0), cv (13.7), v (33.0), and f (56.7) by classifying the male offspring of *y cv v f/l* females. These tests showed that ten of the eleven lethal chromosomes had their lethal effect localized in one, and only one, of the three regions marked by *y cv v f*. One lethal chromosome, *lr 30,* behaved differently as will be shown below.

If the lethal action of a chromosome is restricted to a single crossover region it is, of course, still possible that more than one lethal locus is present within this region. The existence of two (or more) lethals would usually result in an apparent reduction of recombination between the two marker genes (as determined from the male offspring) since all male zygotes with crossovers between the lethals would die. Normal, or increased recombination values between the two marker genes of a lethal region were, therefore, considered as indicators of the presence of a single lethal only, or at least lethals so closely linked that little crossing over occurred between them. When decreased recombination values between the marker genes were observed the same genes were sometimes followed in test crosses in which the recombination values could be determined from the female offspring. Since the existence of multiple and separable lethals in a region would not reduce the recombination values as determined from the female offspring these tests could show whether the reduction in recombination frequency appeared only from the male offspring or from both sexes. In the former case presence of multiple lethals, in the latter a condition reducing the actual frequency of crossing over in the lethal region was indicated. It should be noted that observed deviations from standard recombination values frequently were not statistically significant although perhaps suggestive.

By means of additional crosses some of the lethal chromosomes were subjected to localization experiments within more narrow limits than those defined by *y cv v f*. The following brief reports on the localization tests list (1) designation of lethal, (2) mean viability index (100 percent doubtful = lethal), (3) region of lethal action, (4) increase or decrease in recombination of marker genes in region of lethal action as determined from female or male offspring (*e.g.,* decrease, ♀ ♀ = decrease determined from female offspring).

(a) Lethal chromosomes with indexes over one.

lc 3. 1.258. *y-spl,* within two units to right of *y*. Decrease, ♀ ♀ (two experiments), ♂ ♂ (three experiments).

lr 12. 1.124. *ct-v,* at about 25. Increase, ♂ ♂.

lc 23. 1.112. *y-w,* within .5 units to right of *y*. Decrease, ♂ ♂.

lc 21. 1.080. *ct-v,* within two units to right of *ct*. Increase, ♂ ♂ (two experiments); decrease, ♀ ♀.

lc 10. 1.034. *y-w,* within .5 units to right of *y*. Decrease, ♂ ♂.

None of the tests suggest the presence of more than a single lethal locus. While the tests cannot exclude the presence of more than one separable lethal locus they demonstrate that such loci would have to be closely linked to each other. Such close linkage is not compatible with an interpretation of the observed viability indexes over one as being spurious in the sense of being caused by the presence of separate lethals with frequent crossing over between them. It is concluded that the observed viability indexes are true indexes of intrinsic properties of heterozygous lethals.

(b) Lethal chromosomes with low viability indexes.

lr 30. .633. *y-cv-v,* crossing over *y-cv* reduced to one percent, *cv-v,* reduced to 11 percent. Salivary gland chromosomes show an inversion from 4 B/C to 8 C with possibly a short deficiency within the inversion.

lc 4. .778. *v-f,* within one unit to left of *f*. Decrease, ♀ ♀, ♂ ♂.

lc 12. .861. *v-f,* at about 37. Decrease, ♀ ♀, ♂ ♂.

The lethality of the three chromosomes with low viability indexes is either due to a single locus or to closely or absolutely linked lethal loci.

(c) Lethal chromosomes with intermediate viability indexes.

lc 22. .925. *cv-v,* at about 27. Increase, ♂ ♂.

lc 1. .947. *cv-v,* within one unit to right of *cv*. Increase, ♂ ♂.

lr 50. .952. *v-f,* at about 45. Decrease, ♂ ♂.

The data on *lc 22* and *lc 1* are compatible with the existence of single lethals. The third lethal stock, *lr 50,* may possibly have contained a double lethal.

The possibility cannot be excluded that at the time of the viability tests some lethal stocks contained a mixture of chromosomes with a single and with more than one lethal, but that at the somewhat later date of the localization tests only chromosomes with a single lethal were encountered. It is, however, unlikely that this should have happened in every one of the eleven cases in which localization tests were made. On the other hand, the low homogeneity probabilities encountered in repeated tests for viability indexes of two stocks (*lc 21* and *lc 77*) may have been in part caused by the presence of more than one lethal in some, but not all chromosomes. The initial test of *lc 21* with its high index 1.31 is particularly suggestive in this respect.

Lethals with morphological effects in heterozygotes

As indicated earlier, two of the total of 77 lethals studied were found to cause morphological abnormalities in heterozygous females. Both greatly reduce the viability of heterozygotes.

lr 70. The morphological penetrance and expressivity of this lethal are variable. It affects the wing, by shortening, blistering, and causing abnormalities of the veins. Localization experiments place *lr 70* between *v* and *f*, and more specifically in the region which includes *g* (44.4) and *sd* (51.5). The phenotype and localization suggest allelism with *Bag* (*Bg*, 51.6) as described in BRIDGES and BREHME (1944). A considerable reduction of recombination was observed both in the region occupied by the lethal and adjacent to it. Inspection of salivary gland nuclei showed normal chromosomes.

The frequency of aberrant flies depends on temperature. At 26°C Muller-5/*lr 70* females gave an average of 41 percent morphological penetrance; at 17°C this penetrance was only four percent. Moreover, the expression of *lr 70* at the higher temperature was more extreme than at the lower temperature. Concomitantly with the morphological penetrance the viability index for *lr 70* varied with temperature. The initial test, at 26°C, gave an index of .452, the lowest index in our series. A retest at 26°C furnished the value of .490. A test at 17°C gave the very significantly higher index of .869. For each index all doubtfuls were considered lethal. Thus, the penetrance for lethal action of the heterozygous lethal was greatly decreased by the lower temperature although much less so than the penetrance for its morphological expression.

lc 5. This lethal was Notch, both in phenotype and localization. Heterozygotes for *lc 5* in one X-chromosome, and *w spl* in the other exhibit the effect of *spl* but not of *w*. Muller-5/*lc 5* females all show the slight delta-like widening of venation typical for Notch. At about 26°C only 46 percent of the heterozygotes showed other effects on the wings, usually slight notching or blistering.

In the initial test the viability index was .665. A retest gave .613.

In *lc 5* the number of sterile test cultures was unusually high, and obviously due to the early death of some of the test females which were found lying on the surface of the food only a few days after they had been collected. In order to analyze the cause of these deaths, females of the constitution Muller-5/*lc 5* or Muller-5/+ which had been stored for a variable number of days before being placed into the test cultures were separated into groups based on the length of the storage period. Then the frequency of sterile cultures was determined for each group. It was found that sterility of 403 females stored for less than two days amounted to 25 percent; while sterility of 895 females stored for two or more days declined to four percent, that is, the 895 females were those which had lived two or more days in storage before they were used in matings. It may be assumed that most of the 25 percent sterile females belonged to that half of the tested flies which were lethal-carriers. The sterility of lethal-carriers in this experiment would thus amount to 50 percent. In another test, involving 217 Muller-5/*lc 5* females from stock, 26 percent had died within the first two days. It thus appears that the low viability of *lc 5* carriers is perhaps wholly due to sterility and early death of the imagos.

Direct viability tests using visible marker genes

Among the lethals whose loci were determined there were two, *lc 23* and *lc 10*, which gave considerably less than one percent recombination with *y*. Using a *y*-carrying X-chromosome which except for a terminal section to the left of the *w* locus was derived from Canton-S, females of the constitution

$y+/+ lc\ 23$ and $y+/+ lc\ 10$ were obtained and mated to y males. The F_1 females consisted of the two classes $y+/+ l$ and y/y (plus rare crossovers). The ratio between these two types furnishes a viability index for the heterozygous lethal genotype (a) provided an adjustment is made for the simultaneous difference in heterozygosity *versus* homozygosity for y, and (b) provided that no interaction of viability between the y and lethal loci occurs. The crosses were done in mass cultures, as described in " Methods " for the F_1 generation of crosses (page 415) except that for this experiment several precautions were taken in order to reduce the number of variables between bottles. The food for all bottles came from the same batch, and 60.0 grams were used for each bottle. In addition, one-half sheet of Kleenex was partially submerged in the food, and one drop of a suspension of live yeast in water was put on the food. The parents for each test were collected from bottles which had been started at the same time. Females for all tests had emerged during the same seven-hour period, and were put in the culture bottles with y males when they were about 1.5 to 8.5 hours old. All the y males were collected from the same set of bottles at the same time and were 0–4 days old when they were put in the culture bottles. Forty males and forty females were used for each bottle. Five bottles were prepared for each lethal and six for the control. Cheesecloth bottle coverings were used so that, because of dryness, few pupae would form on the upper part of the bottle. Four days after parents had been put into bottles, they were removed within the same 45-minute period for all 16 bottles. The number of female parents which had survived four days ranged from 37–40 (average 38) for the control, 38–40 (average 39) for *lc 10*, and 31–39 (average 36) for *lc 23*. The number of male parents was 34–40 for the control, 27–38 for *lc 10*, and 35–40 for *lc 23*. After the parents were removed, yeasted paper was added to all bottles within one 20-minute period. One and one-half sheets of Kleenex were soaked in a yeast-water mixture (50.0 g wet yeast in 300 cc water), then squeezed almost dry, formed into a globular wad, and partially submerged in the central portion of the food. This very favorable food supply permitted development of large numbers of offspring. In order to avoid losses during collection of the offspring, the bottles were not shaken out in the usual way, but the flies were collected by means of an aspirator, from the ninth through the nineteenth day.

In the control experiment, among the offspring of $y/+$ females $\times$ y males the ratio of $y/+$ to y/y females ($n = 6109$) was .9751. This was nearly identical with the ratio of + to y males, .9756 ($n = 6069$). The unadjusted ratio of $y+/+ lc\ 23$ to y/y females was .9755 ($n = 4587$) while that of $y+/+ lc\ 10$ to y/y females was 1.0110 ($n = 5126$). Adjusting the indexes for the lethals to that of the control, and using the indexes carried out to six significant figures (lethal index/control index), the adjusted indexes for *lc 23* and *lc 10* become 1.0004 and 1.0368, respectively.

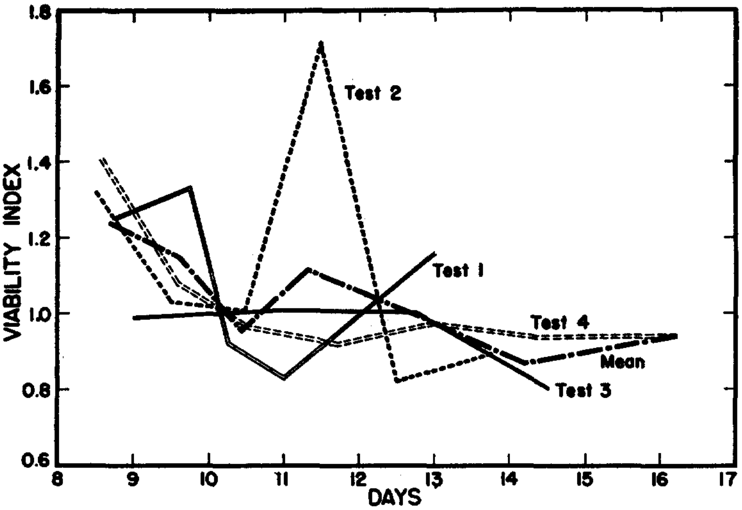
Both indexes are above one, and in this regard agree with the indexes obtained earlier for the two lethals by progeny tests. With *lc 10* there is also close quantitative agreement for the viability indexes obtained by direct classification (1.0368) and progeny test (1.034). For *lc 23* the agreement is poorer, namely 1.0004 *versus* 1.112. This difference may be partly due to differences between the chromosomes used in the two kinds of tests. To some degree the difference seems also to be based on a peculiarity of the emergence pattern of heterozygotes for *lc 23*. As will be shown in the next section, in both direct and progeny tests the frequencies of *lc 23* heterozygotes ($y+/+$ *lc 23* and Muller-5/*lc 23*) relative to non-lethal carriers were higher during the first two days of adult emergence than during the following days. Since, in the progeny tests, emerging females were usually collected for a shorter period than in the direct tests where all flies were counted, it is likely that the higher viability index obtained in the progeny tests is a reflection of selection for testing of early emerging flies. The high experimental progeny test "viability index" for *lc 23* must therefore be redefined as an index of increased speed of development exhibited by females heterozygous for this lethal.

It may be added here that *lc 23* is a lethal which in localization, and phenotypic effect in hemizygous males, seems to correspond to the well known tumor-causing *l(1)7* discovered by BRIDGES. A review of work done on it is given in BRIDGES and BREHME (1944). It thus appears that *lc 23* causes tumors and larval death in hemizygotes, but increased speed of development in heterozygotes.

Relative rates of emergence from the puparium

For some of the experiments all Muller-5/+ and Muller-5/*l* females emerging from puparia in a set of culture bottles were collected. In other experiments only a portion of the total number of females was collected. Whenever only a portion was used, the collections were made at various times during the period when adults were emerging in a set of culture bottles; and in most of these experiments a high proportion of the females were collected within the first four days that adults emerged. Although more than half the total number of flies that could be collected from a bottle emerged during the first four days, it was considered important to investigate whether the proportion of emerging lethal-bearing to lethal-free females, that is, the viability index, remained constant from day to day or whether significant deviations occurred. Such deviations would be expected if, for instance, the time of development from egg to emergence from the puparium differed in lethal-bearing as compared to lethal-free females, or if the change of cultural conditions with time acted selectively on the egg, larval, or pupal survival of the two genotypes.

In numerous cases records of date of emergence were kept for the females tested. Daily viability indexes were calculated for 11 lethals which had had large numbers of flies tested, including the three with best established total indexes over one, *lc 23*, *lr 12*, and *lc 3*, as well as the two lethals with visible effects. The daily indexes of eight lethals did not show any persistent trend. Lethal *lc 21* showed initially low, followed by high indexes; and *lc 3* exhibited in a more variable way a similar pattern suggesting retarded development. The last lethal, *lc 23*, showed a particularly interesting pattern of changing indexes, namely, a considerable surplus of lethal-bearing heterozygotes during the first days of emergence, and a slight deficiency in later days (figure 5). This pattern of increased speed of development was found in three separate series of tests including both progeny tests and direct ones. The only striking exception found in these three was a second rise of the index on the twelfth day in one of the test series. A fourth test showed no marked difference from the control until the last collection when there was a sharp decrease in the proportion of lethal-carrying females.



RANK	1	2	3	4	5	6	7	TOTAL
Test 1	124	203	119	108	95	–	–	649
Test 2	188	262	149	122	80	83	–	884
Test 3	967	–	–	725	114	133	–	1939
Test 4	96	893	1031	1128	685	548	206	4587
Mean	1375	1358	1299	2083	974	764	206	8059

FIGURE 5.—Emergence rates: A comparison of females carrying an *lc 23* X-chromosome and females with other types of X-chromosomes. Both types of females which were compared in a test came from the same mass cultures, and had the same parents. Flies were collected at intervals of about 24 hours. Each point marks the viability index for all the flies collected during an interval and the mid-point of the time interval. Rank indicates the sequence of collections, and the rows show the numbers of flies collected for each test. Time was measured in days after the parents were put in the culture bottles. Types of X-chromosomes compared: Tests 1 and 2, *lc 23*/Muller-5 ÷ +/Muller-5; Test 3, *lc 23/y cv v f* ÷ *y/y cv v f*; Test 4, *lc 23/y* ÷ *y/y*.

For a number of lethals, data are available which show emergence rates of Muller-5/*l* relative to Muller-5/Muller-5 females (figure 6). Based on large numbers the rates differ strikingly from one lethal to another. Since the variability within the set of cultures for each lethal was rather small, it appears thus that many lethals have characteristic patterns of emergence which distinguish them from one another.

The findings on specific rates of emergence of heterozygotes for lethals have a bearing on the interpretation of the viability indexes. In experiments in which all females produced throughout the entire emergence period by F_1 $l/+$ mothers were tested for their genotype the viability indexes are strictly indicators of viability of the two types of F_2 females, regardless of different rates of emergence. When, however, the F_2 females were collected over part of the emergence period only; then the proportions of the two types may be a reflection of the different emergence patterns rather than of true viability. This has been pointed out earlier for *lc 23* whose viability index over one was "redefined as an index of increased speed of development." It is likely that other indexes, over one or below one, also are subject to redefinition in terms of

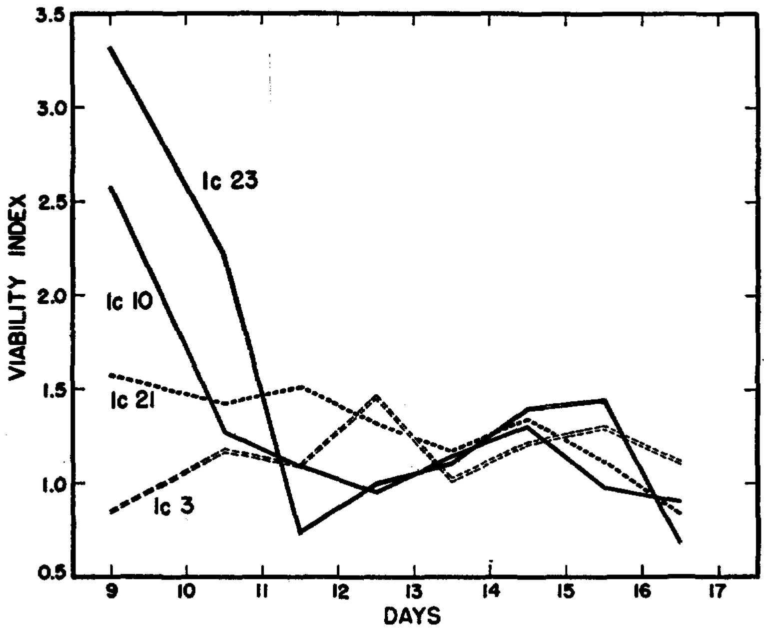


FIGURE 6.—Emergence rates: A comparison between females with both X-chromosomes Muller-5 and females with one Muller-5 and one lethal-bearing X-chromosome. Both types of females which were compared in a test came from the same mass cultures, and had the same parents. Flies were collected at intervals of about 24 hours. Each point marks the viability index for all the flies collected during an interval and the mid-point of the time interval. Time was measured in days after the parents were put in the culture bottles. The numbers of X-chromosomes compared in each test: *lc 23*, 4720; *lc 21*, 4075; *lc 10*, 3310; *lc 3*, 4416. (Viability Index $\doteq$ M-5/l $\div$ M-5/M-5.)

developmental rates. From the point of survival in natural or artificial populations, relative developmental rates are probably often more significant than more static viability differences.

The relativity of viability indexes

Viability indexes are not absolute measures of the capacities of specific genotypes. Obviously, variations in environment and genetic background would be expected and are known to affect viability. The viability indexes determined in the present study imply an additional variable. Even apart from the influence of relative rates of emergence they do not simply represent the frequency of survival from egg to reproductive period of lethal-carriers in relation to a standard of viability as determined in independent experiments. Rather, the indexes given constitute relative frequencies of lethal-carriers and lethal-free females developing in the same culture bottles. The frequency of lethal-free females in these cultures could be regarded as a general standard, if it could be assumed that the development of the lethal-free females proceeded without being affected by the presence of other genotypes. In reality, this assumption is not justified.

In order to study the problem of possible interaction in viability of different genotypes developing in the same culture, three lethals, *lc 3, lc 10,* and *lc 21,* were selected. All three lethals increased the viability of heterozygotes. F_1 females from the cross, $+/l$ female × Muller-5 male, were mated to Muller-5 males, and single females with three males were placed in creamers. Comparisons were then made between the numbers and types of flies hatched in cultures whose parent females were Muller-5/+ and those which were Muller-5/*l*.

TABLE 7

Mean numbers of offspring in various classes from Muller-5/non-lethal and Muller-5/lethal females × Muller-5 males.

Lethal strain	P ♀ Muller-5/non-lethal						P ♀ Muller-5/lethal				
	No. of cultures	M-5/+ ♀♀	M-5/M-5 ♀♀	M-5 ♂♂	+ ♂♂	Total	No. of cultures	M-5/l ♀♀	M-5/M-5 ♀♀	M-5 ♂♂	Total
lc 3	54	28.4	20.1	23.4	27.8	99.6	21	29.9	22.2	24.7	76.8
lc 10	47	30.7	20.6	21.0	29.1	101.5	37	34.4	26.3	28.6	89.4
lc 21	24	29.0	16.7	19.6	31.0	96.3	20	32.0	23.4	24.9	80.3

The data are summarized in table 7. On the average, the yield of cultures free from lethals was higher than from lethal-carrying cultures. A higher yield was to be expected if it were true that the presence of the lethal male genotypes and of the female lethal-carriers did not affect the development to imagos of the other genotypes. Specifically, under these circumstances the total number of flies hatched in the lethal-carrying cultures should have been approximately 25 percent less than in the lethal-free cultures, the deficiency being accounted for by the death of the male lethal zygotes (the death of a fraction of the female lethal carriers, as well as other genotypes being disregarded). However, contrary to this specific expectation the reduction in total number of flies emerging in lethal-carrying cultures amounted to only 23, 12, and 17 percent for *lc 3, lc 10,* and *lc 21* respectively. All three surviving classes of flies account for this relatively higher yield, but in these experiments the replacement of the lethal genotypes was not complete. Otherwise the yield of the lethal-carrying cultures would have equalled that of the lethal-free ones. Moreover the amount of replacement differed for the three lethals tested. These facts indicate dependence of successful development of non-lethal genotypes on presence or absence of simultaneously present lethal genotypes. Apparently, a surplus of eggs is laid in the lethal-carrying cultures, and presumably in the lethal-free cultures; but the death of lethal genotypes provides room for the development of greater numbers of the three nonlethal genotypes in the lethal-carrying cultures than was possible in the lethal-free cultures.

Under different circumstances replacement was found to be absent, or on the contrary to reach 100 percent: (1) Absence of replacement was encountered in the direct viability tests described in a preceding section. The average yield of six cultures with $y/+ \times y$ parents was 2029.7; while the average yields of five cultures each of $y+/+$ *lc 23* or $y+/+$ *lc 10* $\times y$ parents were only 1378.0 and 1512.8. Therefore there was 32 percent reduction for *lc 23* and 25 percent reduction for *lc 10* where 25 percent reduction was expected for both if there were no replacement. (2) Very high replacement was observed in other experiments with *lc 3* and *lc 23* (table 8) in which the yield of $+/l$ females was compared to that of control $+/+$ females, both mated to Muller-5 males. The culture methods used were the same as those described earlier (p. 440) ex-

TABLE 8

Numbers of offspring and sex-ratios from +/+ × Muller-5 and +/lethal × Muller-5. For details see text.

P ♀	Culture no.	Total F_1	♀♀/♂♂
+/+	1	1481	0.87
	2	1541	1.09
	Total	3022	0.97
+/lc 3	1	1281	2.08
	2	1402	1.86
	Total	2683	1.96
+/lc 23	1	1447	1.98
	2	1682	1.99
	Total	3129	1.99

cept for the following changes. For each lethal and for a control two bottles were used. Female parents were all collected within the same 13-hour period. The females and males were put in the culture bottles when the females were 17–30 hours old and the males were 0–2 days old. The parents were left in the culture bottles for five days. The numbers of parents surviving after five days were not recorded. Two pieces of Kleenex were used instead of one and one-half. Collections of flies were made through the seventeenth day.

The three sets of bottles showed the expected proportions of female to male flies, namely, approximately 2 ♀♀ : 1 ♂ in the lethal-carrying bottles and 1 ♀ : 1 ♂ in the lethal-free bottles. However, in spite of the death of about one-half of the male zygotes in the lethal-carrying bottles their total yield was only 11 percent less in the *lc 3* bottles than in the lethal-free cultures, and even 3.5 percent more in the *lc 23* bottles.

The existence of the replacement phenomenon accentuates the effect of intrinsic viability differences between the heterozygotes of the different lethals studied and lethal-free females; an intrinsically lower viability of lethal-carriers than of lethal-free females may result not only in a relative decrease of the former but also in an absolute increase of the latter. Conversely, an intrinsically higher viability of lethal-carriers may not only mean a relative but also an absolute increase of this class. Thus, the different viability indexes obtained in the present study are functions of intrinsic viability differences, of overpopulation, and of competition in the cultures. It is likely that all these factors play a role in natural populations of developing flies. Furthermore, as has been discussed in the preceding section, some of the viability indexes may not be related to viability *per se* but be reflections of specific emergence patterns of lethal heterozygotes.

DISCUSSION

The data presented in this paper have shown that the majority of, if not all, so-called recessive lethals in the X-chromosome of *Drosophila melanogaster* have a dominant effect on viability. Most of the lethals depress viability in heterozygotes as compared to lethal-free females. A few lethals increase viability in heterozygotes. The range of viability indexes of the 75 lethals without morphological effect in heterozygotes extends from about 1.3 to .6. The addition of lethals with variable morphological penetrance in heterozygotes extends the range down to .45.

For the 75 lethals a mean decrease of heterozygote viability of the order of four percent has been calculated. Since this average lumps together lethals with decrease, neutrality (if it exists), and increase of heterozygote viability, it is perhaps more informative to state that most lethals decrease heterozygote viability, and that the observed average decrease caused by these lethals is approximately four percent. A more accurate determination of this value is not available since it is (1) subject to a considerable sampling error, and (2) would depend on an exact, but lacking, knowledge of how many, and which lethals have no effect, or an improved effect on heterozygote viability. The estimate of approximately four percent average decrease is remarkably similar to a value reported by MULLER and CAMPBELL (MULLER 1950a, b, c) for autosomal lethals, induced by ultra-violet light.

The discovery of lethals which increase the viability of heterozygotes over that of lethal-free homozygotes adds further to the now known number of cases with monohybrid heterosis. It is, of course, possible that the increases in viability described in this paper are the results of specific interactions of the heterozygous lethal locus and the complex heterozygosity present in the X-chromosomes at other loci (Muller-5/l), as well as with the undefined heterozygosity in the autosomes. Monohybrid heterosis, however, is always developmentally linked up with genic interaction at different loci. While it is possible and even probable that the superiority of the lethal heterozygotes may occur in some genetic backgrounds and not in others, it remains very probable that the difference in the constitution of the lethal locus is the decisive differential.

A possible objection to this statement could be raised. Should the lethal-carrying X-chromosomes contain genes other than the lethal which would tend to increase the viability of the heterozygote; then the observed viability would not be due to the lethal locus itself. We have no data at present with which to check this point rigorously. It can be stated, however, that such genes would have to be located rather close to the lethal locus in order to produce an observable effect. This is so because there is opportunity for free crossing over between the lethal-carrying and the lethal-free Canton-S chromosomes of the *l*/+ females whose daughters furnish the Muller-5/*l* and Muller-5/+ test females.

The existence of deleterious genes with increased viability in heterozygotes in a population should lead to a higher accumulation of the deleterious allele than would be expected from its ill effects in the homozygote. For the sex-linked lethals described in this report the increased accumulation would be negligible since selection against the lethals in the hemizygote would by far outweigh any positive selection for the heterozygote. However, for autosomal lethals the accumulation effect might be appreciable. This phenomenon has taken on significance in human genetics as a possible explanation for the high allele frequencies for deleterious genes such as those for Cooley's anemia (NEEL 1950).

In recent years the literature on the effect of heterozygotes on viability and on other traits has been reviewed repeatedly. MULLER (1950b, c) has commented on the studies in Drosophila by MASING (1938, 1939), DOBZHANSKY and WRIGHT (1941), BERG (1942), and DUBININ (1946). GUSTAFSSON and his collaborators (GUSTAFSSON 1946, 1947; GUSTAFSSON and NYBOM 1950; NYBOM 1950; GUSTAFSSON, NYBOM, and VON WETTSTEIN 1950) have covered the field even more widely and contributed important new data. It is not necessary, therefore, to discuss the relevant literature once more, except to call attention to TEISSIER's significant studies (1942).

The significance from the standpoint of population genetics of decreased viability effects of heterozygotes of deleterious genes has been treated among others by DOBZHANSKY and WRIGHT (1941), STERN and NOVITSKI (1948), and particularly in respect to human populations, by MULLER (1950a, b, c). MULLER has stressed not only the fact that genetic death due to deleterious genes in heterozygotes will be by far more frequent than in homozygotes, but he has also emphasized that a deleterious gene of slight dominance "would also wreak much the greater part of its damage short of death in heterozygotes" (page 131, 1950b). This may well be true in many cases, and has been discussed above with respect to sterility (*lc 3*), early death of an adult (*lc 5*), rate of development (*lc 3* and *lc 21*), and morphological effects (*lr 70* and *lc 5*). However, impairment of carriers does not necessarily occur with all genes. In the development and functioning of an organism there occur frequently threshold phenomena which result in all-or-none effects. The alternative between death and living is not always one between death and impaired living, but may be one between death and normal life.

SUMMARY

1. Seventy-five sex-linked lethals in *Drosophila melanogaster* were tested for viability effects on females heterozygous for a lethal as compared to females not carrying a lethal (viability of these = 1). None of these lethals showed dominant morphological effects. Thirty-six of the lethals had arisen spontaneously in controls, while 39 lethals occurred in males exposed to about 50 r gamma irradiation.

2. On the average the viability of heterozygotes for lethals was .965 as calculated from an overall total of lethal-carrying and lethal-free females. No significant difference in viability was found between lethals of control and irradiated origin.

3. The various lethals have characteristically different viability indexes ranging in initial tests from .602 to 1.312 with the mode in the .925–.974 interval. The best estimate of the mean viability as calculated from the 75 separate indexes was .961, a decrease of viability of approximately four percent per lethal.

4. Statistical considerations, partly based on retests of specific lethals, established with high probability that several lethals increase the viability of carriers beyond that of noncarriers.

5. Spurious viability indexes over one could be obtained if there were more than one lethal per chromosome. Localization tests eliminated this possibility for the lethals so studied.

6. Two lethals with slight, or variable morphological effects in heterozygotes were also investigated. They showed very low viability indexes. For one lethal the low viability appears to be due to early post-imaginal deaths.

7. The viability indexes obtained depend on competitive survival of lethal-free and lethal-carrying individuals growing up together in the same cultures. In some experiments partial or complete replacement of lethal male hemizygotes by viable genotypes has been demonstrated.

8. Some lethals were shown to change the rate of emergence of heterozygous females, either by slowing down or increasing the speed of development. The latter applies to a lethal which, in hemizygous state, produces tumors and may be identical with BRIDGES' *l(1)7*. The viability indexes obtained in this study are reflections not only of differential survival of lethal-carriers, but also of their different rates of development.

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